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Comparative genomics: tracking chromosome evolution in the family Ursidae using reciprocal chromosome painting

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Abstract. The Ursidae family includes eight species, the karyotype of which diverges somewhat, in both chromosome number and morphology, from that of other families in the order Carnivora. The combination of consensus molecular phylogeny and high-resolution trypsin G-banded karyotype analysis has suggested that ancestral chromosomal fissions and at least two fusion events are associated with the development of the different ursid species. Here, we revisit this hypothesis by hybridizing reciprocal chromosome painting probes derived from the giant panda *(Ailuropoda melanoleuca),* domestic cat *(Felis catus),* and man *(Homo sapiens)* to representative bear species karyotypes. Comparative analysis of the different chromosome segment homologies allowed reconstruction of the genomic composition of a putative ancestral bear karyotype based upon the recognition of 39 chromosome segments defined by painting as the **s**mallest **c**onserved **e**volutionary **u**nit **s**egments (pSCEUS) among these species. The different pSCEUS combinations occurring among modern bear species support and extend the postulated sequence of chromosomal rearrangements and provide a framework to propose patterns of genome reorganization among carnivores and other mammal radiations.

The eight modern species of ursid bears can be classified into three groups, each having a different karyotype. The six ursine bear species (black, brown, polar, sun, sloth, and Asiatic black bears) share a nearly identical G-banded karyotype, consisting of mostly acrocentric chromosomes (2n = 74) and referred to as the ursine bear karyotype (Wurster-Hill and Bush, 1980; Nash and O'Brien, 1987). The spectacled bear (*Tremarctos ornatus,* TOR), which diverged from an ancestor with the ursine karyotype (UK) some 6 million years ago, has 2n = 52, largely biarmed, chromosomes (Nash and O'Brien, 1987). The giant panda (*Ailuropoda melanoleuca,* AME) diverged from ancestors of all bear species some 12 million years ago and has 2n = 42 chromosomes, of which all but three are biarmed (O'Brien et al., 1985). A previous high-resolution G-trypsin banding chromosome analysis suggested that the spectacled bear karyotype differs from the ursine bear karyotype by only 11 centric fusions (Nash and O'Brien, 1987). The giant panda's biarmed chromosomes also appear to consist largely of fused acrocentric ursine chromosomes. Due to G-banding differences and the small size of many of the chromosomes, the homology between about half of the ursine bear karyotype and that of the giant panda has not been determined precisely.

A comparison of the three distinct G-banded bear karyotypes with the proposed ancestral carnivore karyotype (ACK), 2n = 44, and the results of the molecular phylogeny of ursids (O'Brien et al., 1985; Nash and O'Brien, 1987; Pecon-Slattery and O'Brien, 1995) have led to the following model for chromosome evolution in the bears. The initial event in ursid evolution

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was a global fragmentation of the ancestral carnivore karyotype into a karyotype with a high chromosome number (2n = 74). Ursine bears retained this 2n = 74 karyotype, whereas the giant panda and spectacled bear karyotypes display multiple derivative centric fusions of ursine acrocentric chromosomes, but in different combinations. The putative ancestral carnivore karyotype—based on chromosome banding comparisons (Dutrillaux and Couturier, 1983; Nash and O'Brien, 1987) and supported by the results of reciprocal chromosome painting (whereby chromosome paint probes from two species are applied to each other) between human (*Homo sapiens,* HSA) and cat (*Felis catus,* FCA) (Rettenberger et al., 1995a; Wienberg et al., 1997)—is similar to the present-day domestic cat karyotype,.

An important strength of chromosome painting is that it permits definition of regions of chromosome conservation among different species based on DNA sequence homology (Wienberg and Stanyon, 1995, 1997; O'Brien et al., 1997). We describe here the chromosomal changes that have occurred during ursid evolution based on the results of reciprocal chromosome painting with probes derived from bivariate fluorescence-activated flow-sorted (FACS) human, cat, and giant panda chromosomes. The resulting knowledge of regions of chromosome homology makes it possible to reconstruct, with remarkable precision, the G-banded karyotype of the now extinct progenitor of all modern bears and to identify the patterns of genomic exchanges that occurred during radiation of the Ursidae family.

Materials and methods

The flow-sorting procedures previously used for human and cat chromosomes (Wienberg et al., 1997) were performed for giant panda chromosomes (the bivariate flow karyotype of the panda is shown in Fig. 1). Metaphase chromosome preparations for probe isolation were derived from primary skin fibroblast cell cultures from the domestic cat (FCA-215); giant panda (AME-2 and AME-13); brown bear, *Ursus arctos* (UAR-1); spectacled bear (TOR-1); and human using procedures described previously (Wienberg et al., 1997)

Metaphase chromosome preparations of cat, panda, and spectacled bear were derived from primary skin fibroblast cell cultures according to standard procedures (Modi et al., 1987). To facilitate chromosome identification and specify the regions painted with different probes, chromosome preparations were G-banded prior to in situ hybridization. Chromosome nomenclature and numbering, for the most part, followed previous reports (Nash and O'Brien, 1987). The smaller acrocentric chromosomes of the ursine karyotype, acrocentric chromosomes 19–30, are difficult to identify reliably by G-banding alone. Consequently, they have been renumbered according to the size of spectacled bear chromosome arms to which they are homologous.

G-banded slides were kept in a 45 °C oven for at least 1 wk prior to hybridization. (Slides kept up to 6 mo at 45 °C gave good hybridization signals.) Prior to in situ hybridization, the slides were destained for 1 min in 3:1 methanol:acetic acid, rinsed twice (1 min each) with distilled water, then rinsed twice in 1 × PBS for 1 min and denatured in 70% formamide, 2 × SSC (pH 7) in a 50-ml Coplin jar for 10 s at 70 °C. This procedure produced both well-defined fluorescent signals and reverse DAPI bands (see Figs. 2 and 3), observed by simply switching fluorescent filter sets, on the same metaphase chromosome spreads.

Fluorescent signals were imaged separately with the appropriate filter set using a Zeiss Axioskop epi-fluorescence microscope equipped with a cooled CCD camera (Photometrics CH250). The digital 8-bit gray scale images were transferred to an Apple Macintosh computer for processing, and the images were merged and pseudocolored using Oncor *Image* software. Final images were printed on a Tektronix Phaser 440 color printer.

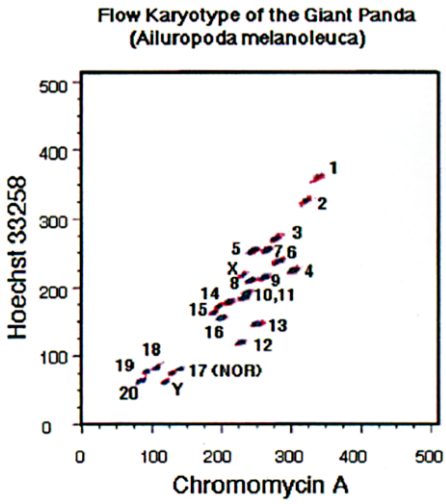

Fig. 1. Bivariate flow karyotype of panda chromosomes from a primary fibroblast culture. Painting probes obtained by DOP-PCR from flow-sorted chromosomes and in situ hybridization to giant panda metaphase chromosome spreads allowed the chromosomal assignment of each peak. Chromosome numbering follows the giant panda karyotype nomenclature (Wurster-Hill and Bush, 1980; Nash and O'Brien, 1987) and as modified in this article.

Results and discussion

Chromosome painting of cat and human probes to giant panda chromosomes

Examples of in situ hybridization of human and feline chromosome painting probes to giant panda metaphase spreads are illustrated in Figs. 2a–d and 3a–d, and the full results are summarized in Fig. 4. Each of the 18 cat chromosome paints is specific for a single cat chromosome, with the exception of D3 and D4, which sorted together; the Y chromosome was not resolved (Wienberg et al., 1997). At least 10 metaphase spreads were analyzed for each painting probe used.

The human Y chromosome painting probe did not hybridize to any panda chromosome. The remaining 23 human painting probes hybridized to all of the panda chromosomes, with the exception of the Y chromosome. Human probes failed to paint two of the panda telomere regions and the proximal quarter of the short arm of panda chromosome 10 (see Fig. 4), as was also observed in primate species (Jauch et al., 1992; Wienberg et al., 1992). The nucleolus organizer region (NOR) of panda chromosome 17 was not painted by any human or cat probe.

The 18 cat chromosome painting probes covered all panda chromosomes, except the Y. Cat probes failed to paint telomere regions in six giant panda chromosome arms, including two (9q and 16q) the human probes failed to paint. Every giant panda chromosome arm except 5q and 8q was hybridized by a single cat painting probe. By contrast, coverage of most giant panda chromosomes required two or more human chromosome paint probes, implying greater segment divergence between human and cat chromosomes than that observed between cat and giant panda chromosomes. A complementary confirmation of the homology segments defined by painting giant panda chromo-

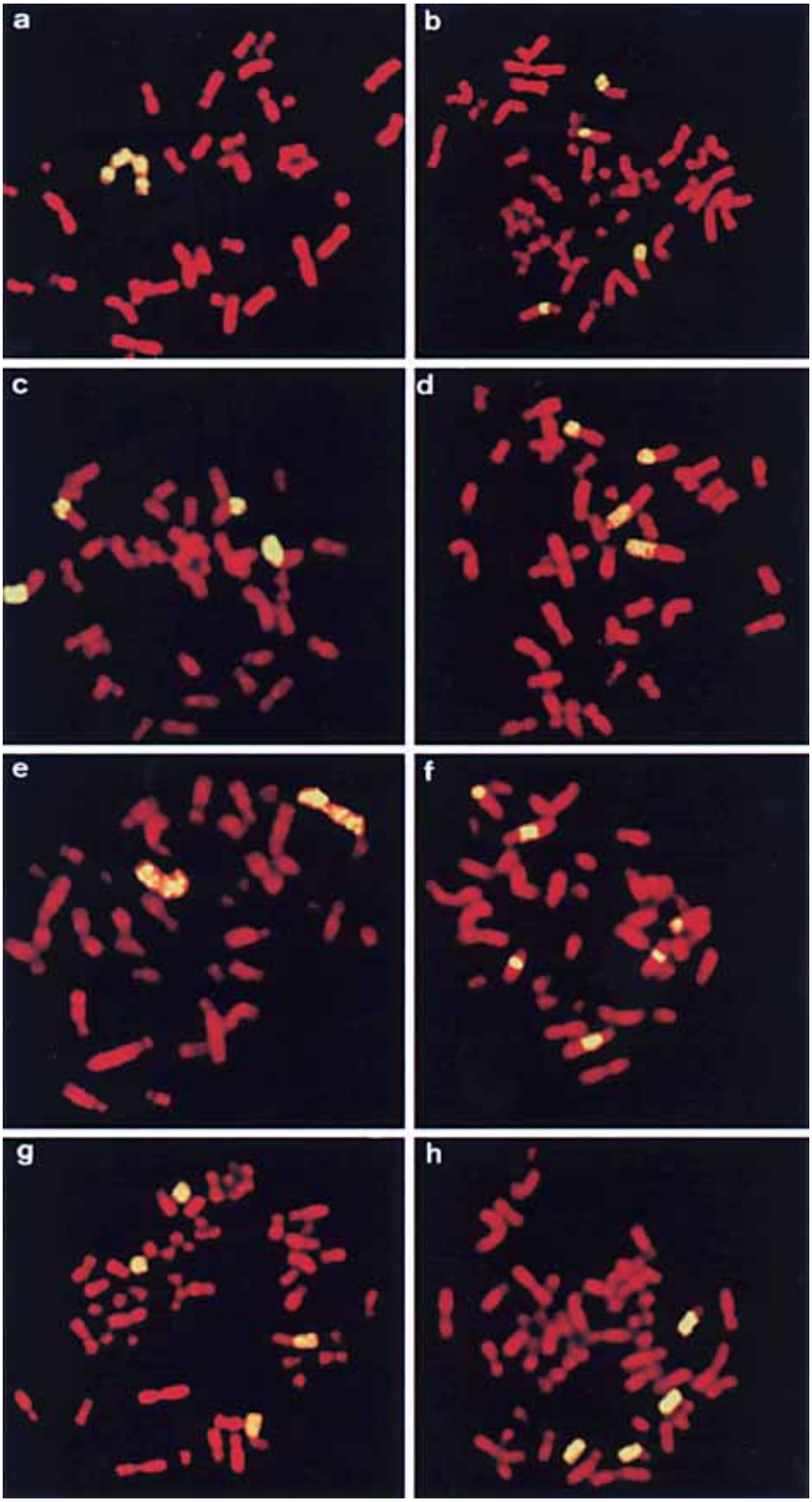

Fig. 2. Examples of in situ hybridization of DOP-PCR generated human, cat, and panda painting probes on panda, cat, and spectacled bear chromosomes: (**a**, **b**) paints derived from human chromosomes 5 and 15 on giant panda chromosomes; (**c**, **d**) paints derived from cat chromosomes A2 and C2 on giant panda chromosomes; (**e**, **f**) paints derived from giant panda chromosomes 2 and 5 on cat chromosomes; (**g**, **h**) paints derived from giant panda chromosomes 7 and 9 on spectacled bear chromosomes.

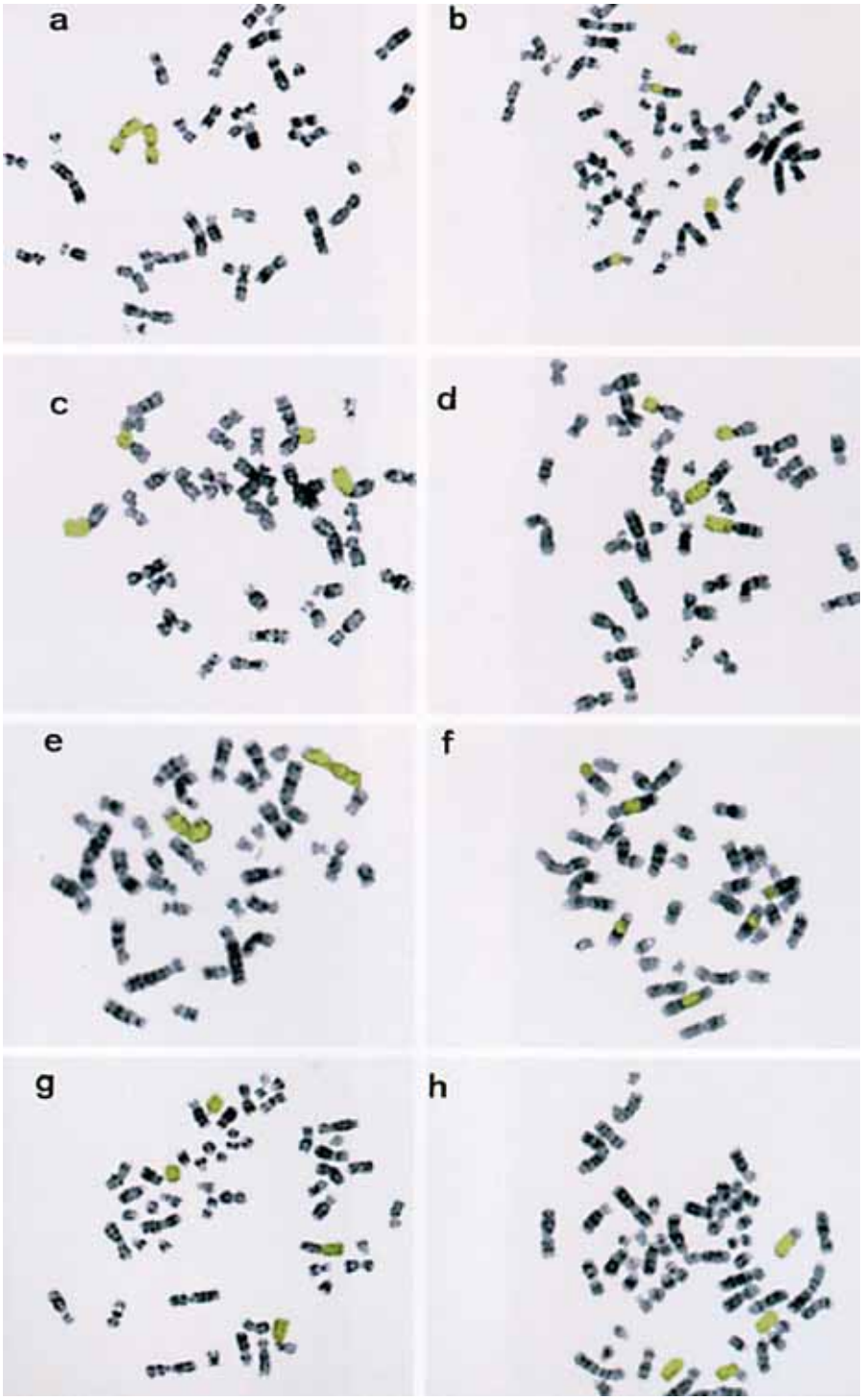

Fig. 3. The same metaphase chromosome spreads shown in Fig. 2 with the hybridization signals superimposed on reverse DAPI-banded chromosomes.

somes (Fig. 4) is the independent results of cat vs. human reciprocal chromosomal painting previously reported (Rettenberger et al., 1995a; Wienberg et al., 1997). In every case, the segment homologies inferred by painting giant panda chromosomes with human and cat chromosome-specific probes (Fig. 4) were in agreement with the previous direct comparison of the latter two species.

The results presented in Fig. 4 form the basis for the identification of the **s**mallest **c**onserved **e**volutionary **u**nit **s**egments (pSCEUS) defined by chromosome painting (O'Brien et al., 1993, 1997) among the compared species. The value of this unit is its interchangeability among different species. The pSCEUS of any number of species hybridized to a reference species can then be compared. All of the species currently ana-

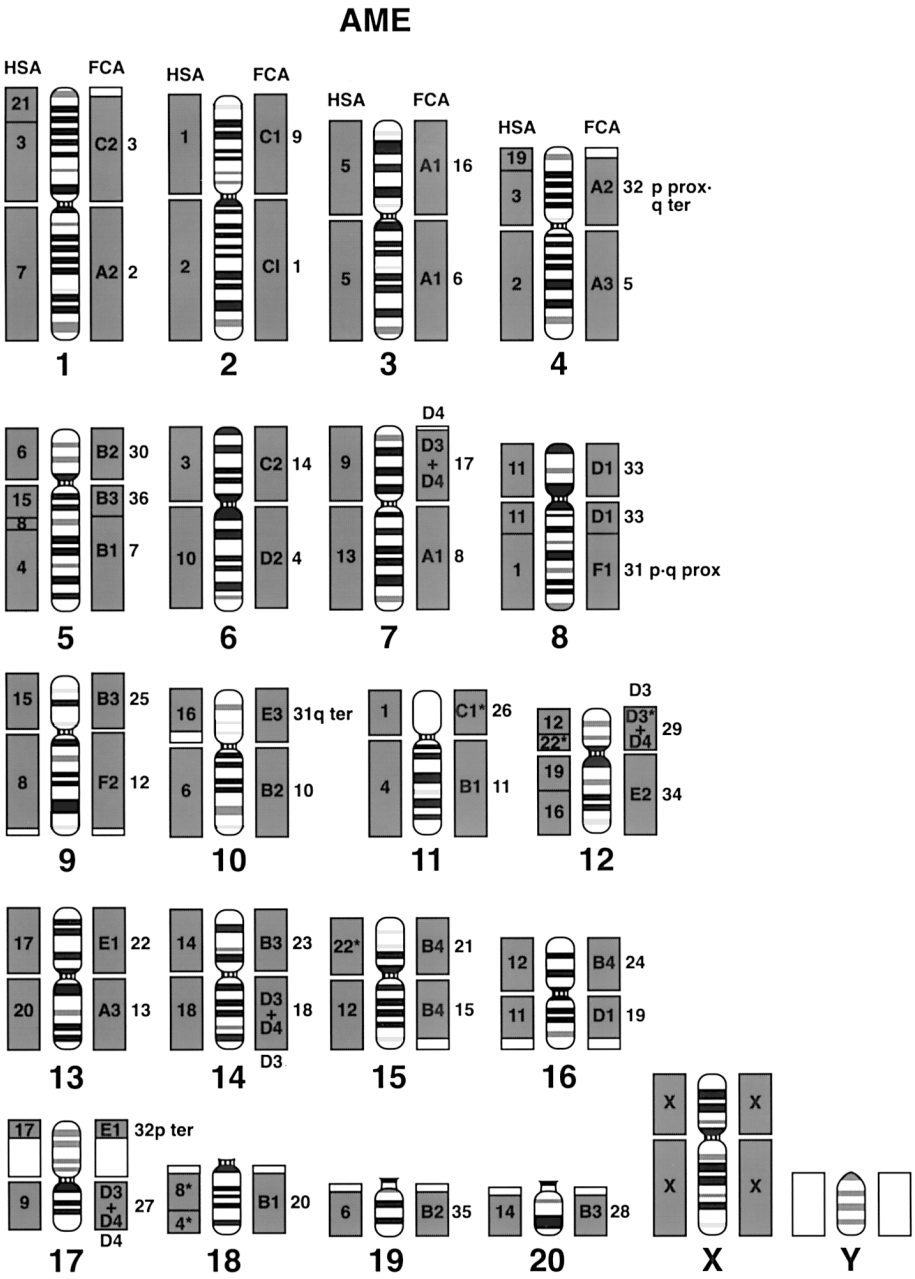

Fig. 4. Hybridization results of human and cat chromosome-specific paints on giant panda chromosomes summarized in a panda ideogram. Giant panda chromosomes 9, 10, 12, 14, 15, and 16 have been renumbered from 10, 9, 16, 12, 14, and 15, respectively, in a previous publication (Nash and O'Brien, 1987). Chromosome numbers were changed to reflect morphological and descending size criteria. The hybridization pattern of human chromosome painting probes on panda is shown to the left of each chromosome; the cat hybridization pattern is shown to the right. Boxes shaded dark gray indicate hybridization, whereas clear boxes indicate the absence of hybridization signal. Since cat chromosomes D3 and D4 were not resolved in the flow karyotype, the hybridization pattern shown for the D3 + D4 boxes was determined by reciprocal painting of panda chromosomes onto cat metaphase chromosomes (see Fig. 5). The number to the right and outside the boxes indicates homologous chromosomes of any of the bear ursine karyotypes (UK). An asterisk indicates very weak signals.

lyzed by ZOO-FISH are linked either by human or cat comparisons and, therefore, can also be compared to any other species in a database.

Chromosome painting of giant panda probes to cat chromosomes

To perform reciprocal painting of giant panda probes on cat chromosomes, bivariate flow sorting was carried out using a chromosomally normal (42,XY) giant panda male cell line. Of the 21 painting probes generated, 20 were specific for single panda chromosomes; panda chromosomes 10 and 11 sorted together (Fig. 1). The results of in situ hybridization of each of these 21 giant panda painting probes to cat metaphase chromosome spreads are summarized in Fig. 5. Representative photographs of hybridizations using giant panda painting probes on cat chromosome spreads are shown in Figs. 2e, f and 3e, f. The hybridization signals produced by the giant panda chromosome paints were less intense on human than on cat chromosomes; however, the human pSCEUS corresponding to ursid segment homologs can be discerned from combined cat/panda and cat/human reciprocal painting. All panda painting probes (except the Y probe) hybridized to all cat chromosomes. The telomere regions of cat D1p and E3p and the entire G-band-negative region of D2p were not painted. In contrast to the reciprocal painting pattern of cat probes on panda chromosomes, where all but two giant panda chromosome areas were

completely painted, eight cat chromosome arms required two or more panda painting probes for complete coverage (Fig. 4).

Chromosome painting of giant panda probes to spectacled bear chromosomes

Giant panda painting probes were hybridized to the spectacled bear karyotype and not to the ursine bears' chromosomes for two reasons. First, the larger, mostly biarmed chromosomes of the spectacled bear are much easier to recognize with reverse DAPI banding than the short, numerous (2n = 74) acrocentrics of the ursine bear karyotype. Second, the spectacled bear banded chromosome arms and ursine bear acrocentric chromosomes are virtually identical (Nash and O'Brien, 1987). In this study ursine bear chromosomes 19–30 (Nash and O'Brien, 1987) have been renumbered according to the size of their G-banded homologs in the spectacled bear (Fig. 6).

The results of in situ hybridization of giant panda painting probes to spectacled bear chromosomes are summarized in Fig. 6. All spectacled bear chromosomes, except for chromosome 23, hybridized with giant panda painting probes. Spectacled bear chromosome 23 bands very lightly with DAPI. This light DAPI staining appeared to be a common feature of all of the chromosome segments that gave a weak or no hybridization signal. The centromere region of all spectacled bear acrocentric chromosomes (Nos. 16–24) were not painted with probes derived from giant panda biarmed chromosomes. If hybridized with a giant panda acrocentric probe, then the whole chromosome was painted (spectacled bear chromosomes 21 and 24 painted by giant panda chromosome 18 and 19 paints, respectively). Except for spectacled bear chromosomes 11 and 12, every chromosome arm was painted by just one giant panda chromosome-specific probe irrespective of whether the giant panda probes were derived from acrocentric or metacentric panda chromosomes.

Although reciprocal hybridization was not performed between panda and spectacled bear (spectacled bear chromosomes were not sorted), it was still possible in most cases to predict by banding homology which giant panda chromosome arm corresponds to a spectacled bear pSCEUS. To verify these predictions and also to determine the few regional painting assignments not resolvable by banding homology, selected cat painting probes were hybridized to spectacled bear chromosomes (Fig. 6). For example, G-banding homology predicts that a giant panda chromosome combination 10q + 11q probe would paint spectacled bear chromosome arms 4q and 3q, respectively. Verification of this prediction is confirmed by the fact that B2qter, which is the cat pSCEUS for panda chromosome arm 10q, paints spectacled bear chromosome 4q, whereas B1qter, the cat pSCEUS for panda chromosome arm 11q, paints spectacled bear 3q (Figs. 4–6).

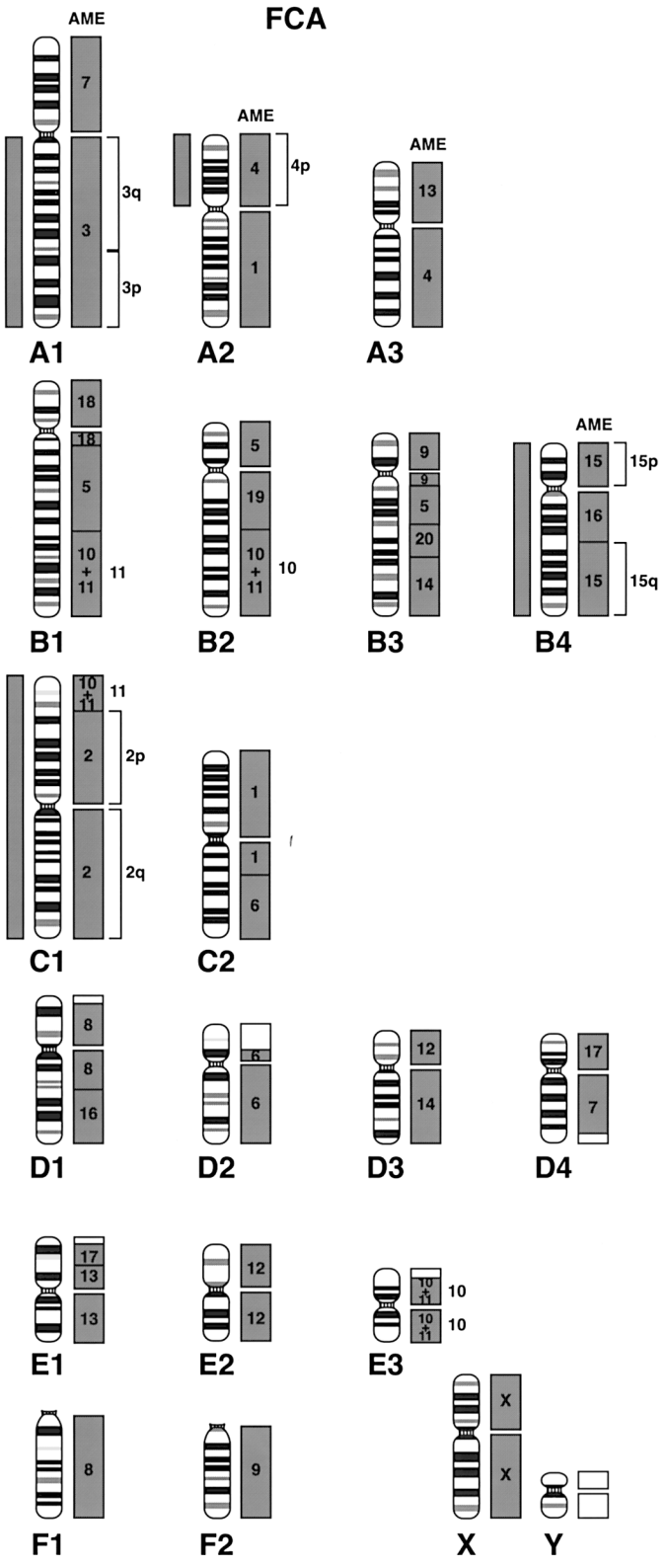

Fig. 5. Hybridization results of giant panda (AME) chromosome-specific paints on cat chromosomes summarized in a cat (FCA) ideogram. The hybridization pattern of panda chromosome painting probes on cat chromosomes is shown to the right of the cat ideogram. Boxes shaded dark gray indicate hybridization with panda probes. Clear boxes indicate no hybridization. Since panda chromosomes 10 and 11 were not resolved in the flow karyotype, the chromosome painting pattern shown for these chromosomes was determined by reciprocal painting of cat chromosomes onto panda chromosomes (see Fig. 2). The solid bars to the left of cat chromosomes highlight ancestral carnivore chromosomes that are preserved in the panda karyotype but not in the spectacled bear or UK. The homologous panda chromosome arm numbers are shown to the right, outside the boxes.

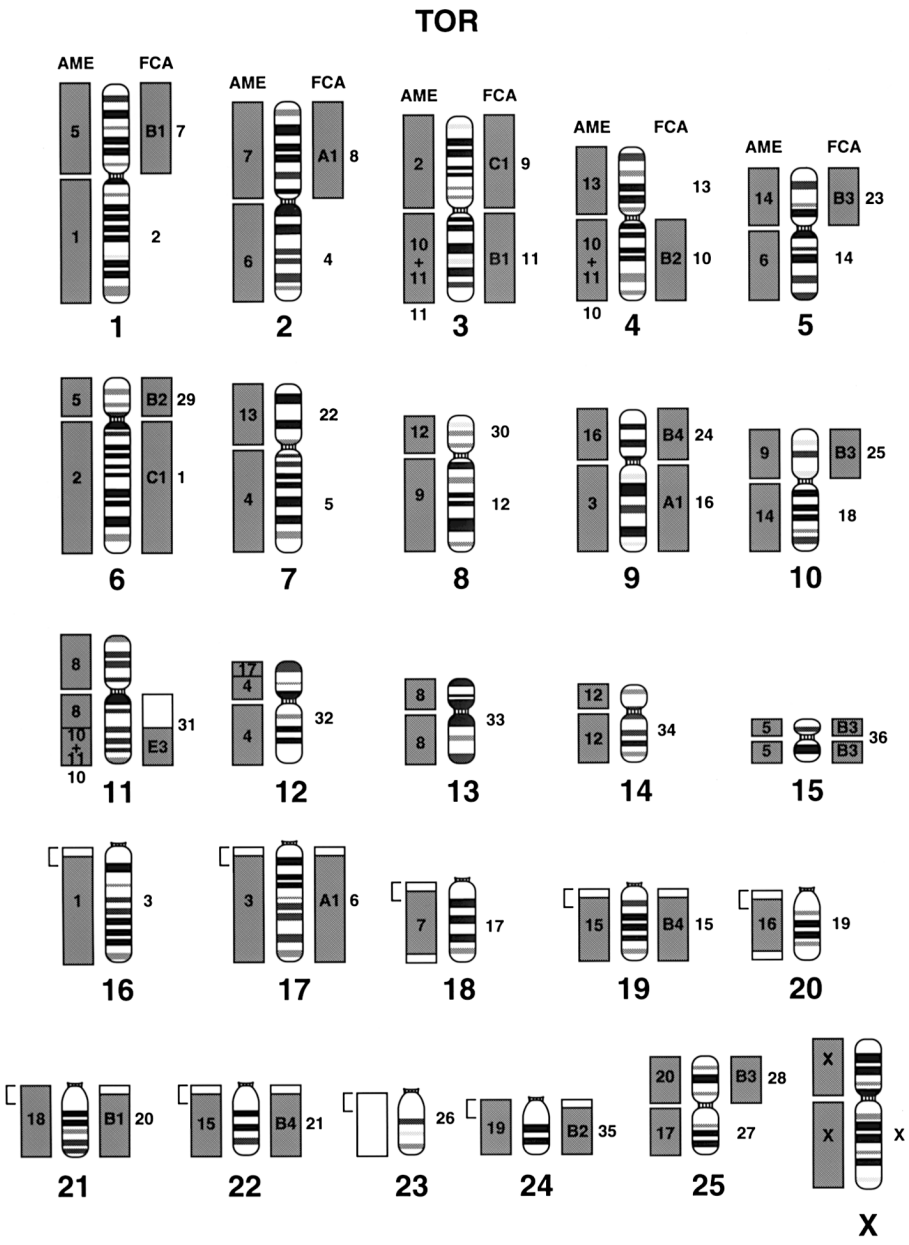

Fig. 6. The hybridization pattern of giant panda (AME) painting probes on spectacled bear (TOR) chromosomes is shown to the left of the spectacled bear ideogram. Selected cat (FCA) chromosome painting probes are to the right. Their use in defining chromosome subregional hybridization patterns between the panda and spectacled bear is described in the text. Boxes shaded gray indicate hybridization, whereas clear boxes indicate no hybridization. The assignment of chromosome 10 or 11 shown above or below the 10 + 11 boxes was determined using cat chromosome pSCEUS homologs to giant panda chromosomes. The numbers to the right and outside the boxes designate ursine karyotype (UK) chromosomes. A minus sign (–) indicates regions that cross-hybridize to panda chromosomes 18, 19, or 20. Paints from biarmed panda chromosomes fail to paint the upper half of this pericentric region.

A second example of how chromosome painting resolves homology between pSCEUS's of different species comes from the examination of giant panda chromosome 5, whose banding pattern alone does not predict the corresponding three pSCEUS's in the spectacled bear karyotype, as it appears to be rearranged. The cat pSCEUS for giant panda chromosome 5 (Figs. 4 and 5) are giant panda 5p/cat B2p, giant panda 5qprox/cat B3qprox, and giant panda 5qter/cat B1qprox. Figure 6 shows that cat chromosome B1 and giant panda chromosome 5 paint spectacled bear chromosome arm 1p, cat chromosome B2 and giant panda chromosome 5 paint spectacled bear chromosome arm 6p, and cat chromosome B3 and giant panda chromosome 5 paint spectacled bear chromosome 15. Therefore, giant panda pSCEUS 5p = spectacled bear pSCEUS 6p, giant panda pSCEUS 5qprox = spectacled bear pSCEUS 15, and giant panda pSCEUS 5qter = spectacled bear pSCEUS 1p. It is possible, therefore, to precisely define all pSCEUS's among human, cat, giant panda, spectacled bear, and, by extrapolation, ursine bear chromosomes without actually performing all of the possible reciprocal paintings.

Similarly, it is possible to distinguish 39 pSCEUS's among ursine, spectacled bears, giant panda, and cat chromosome paints. Each pSCEUS is common to all of these species but is attached differentially, as illustrated in Figs. 4, 5, and 6. In Table 1 we list each of the 39 pSCEUS's and their chromosomal locations in the compared species.

The concordance of gene mapping and reciprocal chromosome painting data between human and cat shows the validity of using ZOO-FISH to establish regional chromosome homologies (O'Brien et al., 1993; Rettenberger et al., 1995a; Wienberg

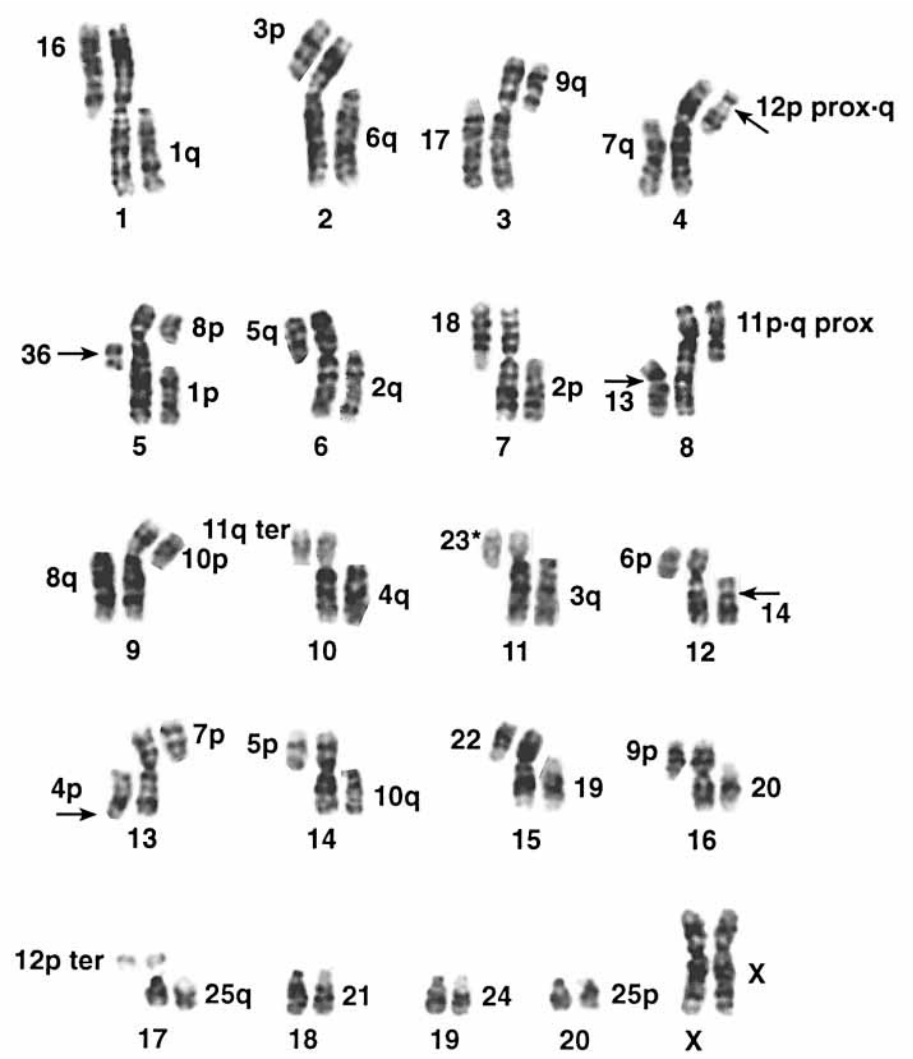

Fig. 7. Comparison of panda and spectacled bear karyotypes. Homologies shown in this figure are based on results of painting of spectacled bear chromosomes with panda and selected cat chromosome-specific probes. Cat probes were used to determine panda/spectacled bear pSCEUS's that were not resolved by panda chromosome paints, as described in the text. Each G-banded panda chromosome is identified by a number or letter directly below it. G-banded spectacled bear chromosome segments are shown to the left and right of each panda chromosome. The arrows show the position of five centromeres that have been displaced in the spectacled bear karyotype relative to the homologous panda chromosomes by an inversion. With the exception of these five inversions, the G-banded patterns of all panda/spectacled bear chromosome arm homologies appear to be the same.

et al., 1997). The correct alignment of human and cat chromosome segments based on giant panda paints (Fig. 4), along with other ZOO-FISH data comparing human with pig, cattle, horse, and deer species (Hayes, 1995; Rettenberger et al., 1995b; Chowdhary et al., 1996; Frönicke et al., 1996; Goureau et al., 1996; Raudsepp et al., 1996; Yang et al., 1997), cat with mink (Hameister et al., 1997), and human with other primates (Wienberg and Stanyon, 1995, 1997), demonstrates the efficacy of painting probes to reveal homologous chromosome segments in different species across the whole mammalian radiation.

Defining the ancestral bear karyotype

In a previous report it was demonstrated by G-banding homology that most of the largest acrocentrics of ursine bears are present in the giant panda karyotype as biarmed fusion chromosomes (Nash and O'Brien, 1987). ZOO-FISH analysis supports and extends these conclusions. The ursine karyotype bear chromosomes homologous to the giant panda and spectacled bear chromosomes are shown in Figs. 4 and 6. Since giant panda chromosomes were used to paint spectacled bear chromosomes (Fig. 6), these results depend on the premise that the spectacled bear karyotype differs from the ursine bear karyotype only in the previously described 11 pairs of acrocentric fusions. In addition to the known G-banding homology among these species, this premise is supported in every case by the fact that a number of painting probes hybridized to both ursine karyotype and spectacled bear chromosomes (data not shown). Figure 7 compares the G-banded chromosomes of the giant panda with those of the spectacled bear based on the homology obtained by chromosome painting. Since panda and spectacled bear exhibit primarily monobrachial (single arm) homology, the matching seen in Fig. 7 requires separating the 11 biarmed chromosomes that distinguish the spectacled bear from the ursine karyotype. Of the 36 ursine karyotype autosomes, 32 are pSCEUS's to whole panda chromosome arms (Table 1). G-banding of these 32 pSCEUS pairs appears to be identical.

It was previously suggested that the original event in Ursidae chromosome evolution was a global fission event from the proposed ancestral carnivore karyotype, with two subsequent independent global fusions, one giving rise to the giant panda and the other to the spectacled bear. By combining chromosome painting and G-banding information, we can test this

Table 1. Chromosome painting–defined small conserved evolutionary unit segments (pSCEUS) in the ursine bear (UK), giant panda (AME), spectacled bear (TOR), and cat (FCA) karyotypes[a]

UK 2n = 74	AME 2n = 42	TOR 2n = 52	FCA 2n = 38
1	2q	6q	C1q
2	1q	1q	A2q
3	1p	16	C2pqc
4	6q	2q	D2
5	4q	7q	A3q
6	3q	17	A1qc
7	5qt	1p	B1qi
8	7q	2p	A1p
9	2p	3p	C1pc
10	10q	4q	B2qt
11	11q	3q	B1qt
12	9q	8q	F2
13	13q	4p	A3p
14	6p	5q	C2qt
15	15q	19	B4qt
16	3p	9q	A1qt
17	7p	18	D4q
18	14q	10q	D3q
19	16q	20	D1qt
20	18	21	B1pqc
21	15p	22	B4p
22	13p	7p	E1pcq
23	14p	5p	B3qt
24	16p	9p	B4qc
25	9p	10p	B3p
26	11p	23	C1pt
27	17q	25q	D4p
28	20q	25p	B3qi
29	12p	6p	D3p
30	5p	8p	B2p
31pqc	8qt	11pqc	F1
31qt	10p	11qt	E3
32pt	17pt	12pt	E1pt
32pcq	4p	12pcq	A2p
33	8pqc	13	D1pqc
34	12q	14	E2
35	19	24	B2qc
36	5qc	15	B3qc
X	X	X	X

[a] Subregional abbreviations: p = entire short arm; q = entire long arm; c = centromere; t = terminus; i = interstitial segment (including neither terminus or the centromere). A chromosome number standing alone means the entire chromosome, from the terminus of the short arm through the terminus of the long arm.

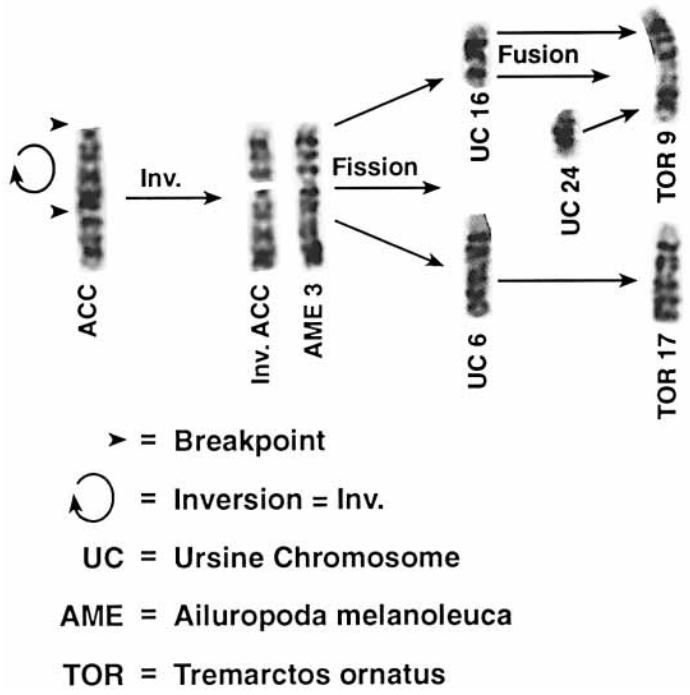

Fig. 8. Pictorial representation of the evolution of an ancestral carnivore karyotype (ACK) chromosome in the family Ursidae. The first step is a pericentric inversion of an ancestral carnivore chromosome (ACC) which internalizes the centromere, forming a biarmed chromosome. This chromosome is rotated 180° and paired with panda chromosome 3, with which it shows complete G-banding homology. It is uncertain whether this chromosome is an acrocentric or a biarmed chromosome in the proposed ancestral bear karyotype. Spectacled bear (TOR) chromosome 9 is shown upside down. Except for the inversion leading to panda chromosome 3, all other rearrangements can be derived by simple fusion and fission events.

hypothesis in greater detail. If we assume that the most common chromosome is primitive or ancestral (Watterson and Guess, 1977), then cat chromosomes A1q, A2p, B4, and C1 are examples of the proposed ancestral carnivore chromosomes (solid bars on the left in Fig. 5). These ancestral carnivore chromosomes are found completely or partially intact in the panda but not in the spectacled bear or ursine bears. Figure 8 illustrates the evolution of one of these ancestral carnivore chromosomes (cat A1q) in the Ursidae. One inversion, a centric fission, and a centric fusion would explain the observed exchanges of modern Ursidae chromosomes. The giant panda retains several ancestral carnivore chromosomes and, like the proposed ancestral carnivore karyotype, has a low chromosome number (2n = 42). This chromosome number may be the ancestral bear disposition as well.

Figure 9 compares the cat and giant panda G-banded karyotypes where homology is based solely on reciprocal chromosome painting results. Notice that the G-banding patterns between the panda chromosome arms and cat chromosome arms or sub-arm regions are remarkably similar. These regions, in many cases, correspond to the pSCEUS defined in Table 1. Since the proposed ancestral carnivore chromosomes (ACC) are largely comparable to the cat karyotype (Dutrillaux and Couturier, 1983; Nash and O'Brien, 1987), and since bear chromosome segments display quite similar G-banded chromosomes to those of the cat, we can conclude the ancestral bear karyotype chromosomes had a similar pattern of chromosome organization.

Inspection of Fig. 9 reveals an asymmetry observed in reciprocal paints between panda and cat. Cat paint probes generally hybridize to the whole arm of giant panda chromosomes, whereas panda paint probes hybridize to cat chromosome fragments. This asymmetry suggests that most giant panda chromosome arms are fragments of the proposed ancestral carnivore chromosomes. To go from the proposed ancestral forms to the giant panda karyotype requires an ancestral bear karyotype

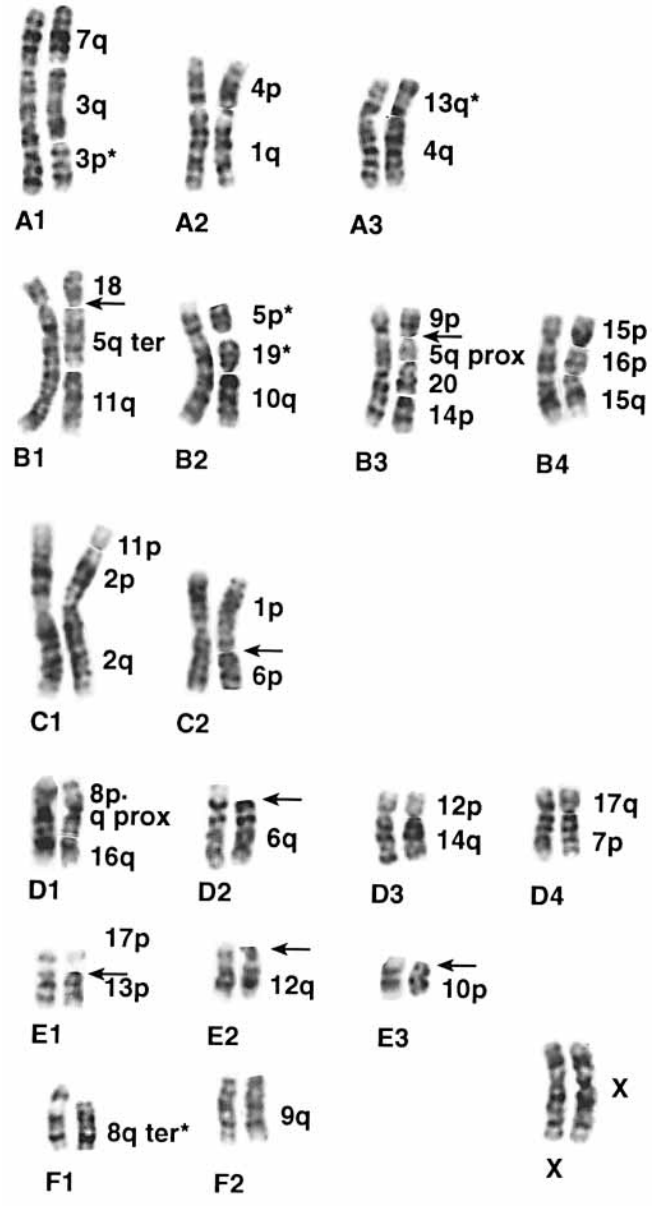

Fig. 9. Comparison of cat with panda karyotypes based on the results of reciprocal chromosome painting summarized in Fig. 5. G-banded cat chromosomes are shown to the left. The homologous panda chromosome segments are shown to the right. Each panda chromosome segment is identified with respect to its orientation in the panda karyotype. Arrows indicate a modified centromere position as compared to the homologous cat chromosome.

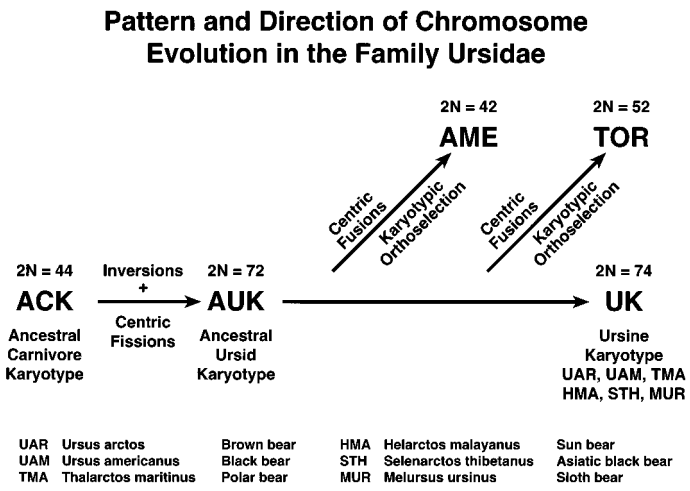

Fig. 10. Pattern and direction of chromosomal evolution in the family Ursidae. The proposed ancestral carnivore karyotype (ACK) consists of 44 chromosomes whose morphology and G-band patterns can be inferred from present-day species that retain these chromosomes. The major chromosomal changes that differentiate the ancestral ursine karyotype (AUK) from ACK involve inversions and centric fusions. Present-day UK bears are chromosomally similar to AUK in number, morphology, and G-band pattern. The panda and spectacled bear karyotypes were derived primarily from the accumulation of independent centric fusions of AUK acrocentrics. Details and the evidence supporting this proposal are discussed in the text.

with a sufficient number of acrocentric chromosomes to reconstruct the panda chromosomes by centric fusions. Based on this presumption, the number of chromosomes in the ancestral bear karyotype would therefore be 2n = 72 (Fig. 9).

The combined information derived from ZOO-FISH and G-banding allows us to reconstruct a rather detailed description of the chromosome evolution of present-day bears from the proposed ancestral carnivore karyotype (Fig. 10). The ancestral bear karyotype consists of 35 pairs of largely acrocentric autosomes (the X chromosomes are the same for the ancestral carnivore and all bears) and was derived from the ancestral carnivore karyotype almost exclusively by inversions and centric fissions. Panda chromosomes 2, 3, 15, 18, 19, and 20 correspond to single chromosomes in the ancestral bear. Panda chromosomes 1, 4, 6, 7, 9, 10, 11, 12, 13, 14, 16, and 17 were derived from the ancestral bear karyotype by successive independent centric fusions of 24 acrocentrics. Extensive chromosomal rearrangement occurring primarily by the accumulation of a single type of rearrangement is called "karyotypic orthoselection" (White, 1975). Karyotypic orthoselection involving centric fissions and fusions is also common in canids (Wayne et al., 1987a, b). Only panda chromosomes 5 and 8 have a slightly more complicated origin. The ursine karyotype, UK (2n = 74), is derived from the ancestral bear karyotype by rather modest changes like those documented for one ancestral chromosome shown in Fig. 8. G-banding indicates that, with the exception of centric fission or fusion, the ursine karyotype and spectacled bear differs from the panda karyotype by only five detectable inversions. The spectacled bear karyotype is a recent independent example of karyotypic orthoselection.

The fact that karyotypic orthoselection involves different kinds of chromosomal rearrangements in different taxa (Baker et al., 1987) suggests a nonrandom mechanism. Some predisposing element or condition in the genomes of these species may have facilitated a particular class of rearrangement. Centric fissions and fusions are prevalent in ursids and canids. It is of interest that acrocentrics in the giant panda, spectacled bear, and perhaps all bears contain cross-hybridizing pericentric sequences not found in biarmed chromosomes. The chromo-

some-specific probes of all panda acrocentrics paint a large pericentric region of all other panda and spectacled bear acrocentrics. Probes from biarmed panda chromosomes paint the distal but not the proximal half of these pericentric regions (see Figs. 2 and 6). The paints for all human acrocentrics also cross-hybridize at the centromeres, and these chromosomes are often found associated at their centromeres in metaphase chromosome spreads (Collins et al., 1991). Recently, we used chromosome painting to analyze chromosome changes involved in the karyotypes of two lemur species (Müller et al., 1997). The karyotypes differed only by Robertsonian fusions, and only fused chromosomes showed cross-hybridization of centromeric heterochromatin.

In summary, karyotypic orthoselection implies a facilitating event in the genome that allows a buildup of the frequency of a given rearrangement in a population necessary for fixation. Repeated accumulation of one kind of rearrangement in a single direction, as seen in the panda and spectacled bear, implies positive selection, perhaps contributed by meiotic imbalance in translocation heterozygotes. It has been suggested that the primary force driving karyotypic orthoselection is the effect rearrangements have on recombination, with higher chromosome numbers leading to increased recombination (for more details of this argument, see Qumsiyeh, 1994). It is as if the genome possesses a built-in capacity to modify chromosome number, such that an increase is triggered by environments characterized by intense selection.

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